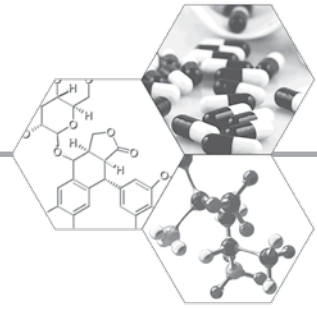

Targeting protein–protein interactions within the cyclic AMP signaling system as a therapeutic strategy for cardiovascular disease

The cAMP signaling system can trigger precise physiological cellular responses that depend on the fidelity of many protein–protein interactions, which act to bring together signaling intermediates at defined locations within cells. In the heart, cAMP participates in the fine control of excitation–contraction coupling, hence, any disregulation of this signaling cascade can lead to cardiac disease. Due to the ubiquitous nature of the cAMP pathway, general inhibitors of cAMP signaling proteins such as PKA, EPAC and PDEs would act non-specifically and universally, increasing the likelihood of serious 'off target' effects. Recent advances in the discovery of peptides and small molecules that disrupt the protein–protein interactions that underpin cellular targeting of cAMP signaling proteins are described and discussed.

Louisa CY Lee[1], Donald H Maurice[2] & George S Baillie*[1]

[1]Institute of Cardiovascular & Medical Science, College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK
[2]Department of Pharmacology & Toxicology, Queen's University, Kingston, Ontario, K7L3N6, Canada
*Author for correspondence:
E-mail: George.Baillie@glasgow.ac.uk

Compartmentalized cAMP signaling

cAMP was discovered in 1956 by Earl Sutherland as the original second messenger molecule that acts to transmit extracellular cues sensed by surface receptors into tangible physiological responses mediated by cAMP effector proteins (reviewed in [1,2]). Since its discovery, cAMP has been the subject of much research and is known to impinge on many essential cellular processes, such as immune function [3], growth [4], differentiation [5], gene expression [6] and metabolism [7].

In a cardiac setting, cAMP has a central role in the orchestration of excitation–contraction coupling, providing sympathetic stimulation following activation of β-adrenergic receptors (reviewed in [8]). Briefly, β-agonists, via binding to β-adrenergic receptors, stimulate the release of the Gαs that binds to membrane-localized AC to trigger the production of cAMP. cAMP can then activate one of three cAMP-effector proteins, PKA, the EPAC or CNGC to evoke a cellular response **(Figure 1)** [9]. PKA can phosphorylate many of the calcium-handling proteins that coordinate excitation–contraction coupling **(Figure 2)**, such as troponin I, RyR2, PLB and L-type calcium channels (LTCCs) causing an increase in the force and frequency of heart beat [10] and an increase in cardiac relaxation [11]. EPAC activation can also alter the dynamics of cardiac calcium flux during excitation–contraction coupling via phospholipase C signals [12,13] and it is suggested that CNGCs may promote chronotropic responses to agents that increase cellular cAMP [14].

One question that has fascinated those interested in cardiac cellular signaling is, how can one ubiquitous signaling molecule exert such a variety of sophisticated actions in the heart? The diffusion of cAMP is rapid (130–700 $\mu m^2\ S^{-1}$) and should rapidly distribute from the outer membrane, through the entire cell, activating all cAMP effectors concomitantly. Groundbreaking work from Brunton and colleagues in the early 1980s reported that several G-protein-coupled receptors could trigger equivalent increases in cAMP but also resulted in each receptor type mediating a specific physiological outcome [15]. Brunton and colleagues proposed that one solution to this puzzle was that the cAMP response and the proteins mediating it were compartmentalized in some way **(Figure 3)**. Further work also proved that physical segregation of PKA within cardiac myocytes resulted in different subsets of PKA substrates being phosphorylated in response to activation of different receptor types [16]. In the field, we now know that the segregation of different PKA pools in cells depends on the scaffolding function of a variety of AKAPs **(Figure 3)** [17]. We also know that cAMP dynamics within heart cells are heavily influenced by cAMP **PDEs**, the only enzyme family that can degrade cAMP by hydrolysis [18]. The development of optical probes, which were able to report real-time, spatial and temporal changes in cAMP showed that spatially restricted cAMP gradients were formed in cardiac myocytes following addition

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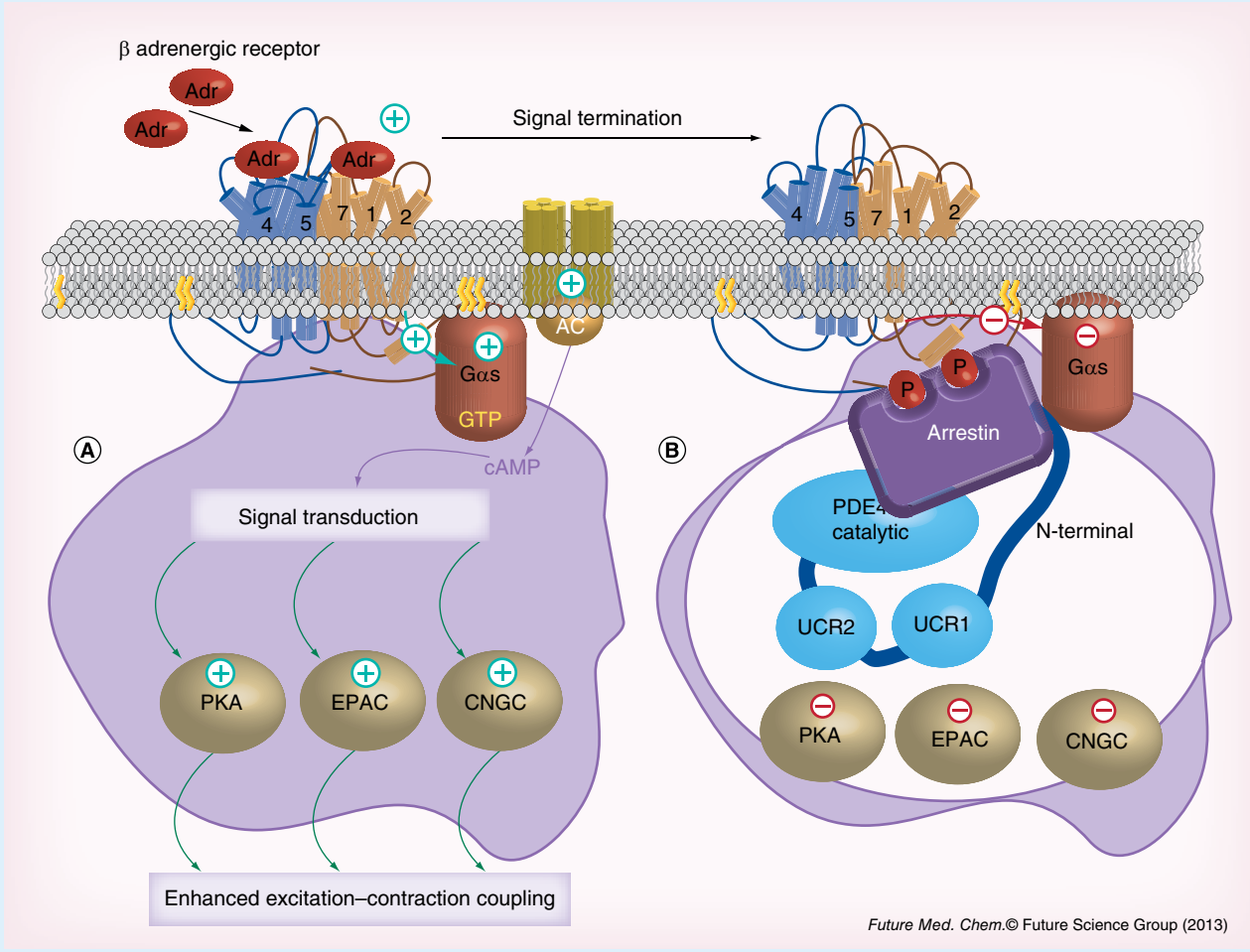

Figure 1. cAMP signaling pathway. (A) Hormones such as adrenaline bind to G-protein-coupled receptors such as the β-adrenergic receptors, stimulate the release of the Gαs that binds to membrane-localized AC to trigger the production of cAMP. cAMP can then activate one of only three cAMP-effector proteins, PKA, the EPAC or cyclic nucleotide-gated ion channels to promote downstream signaling that promotes sympathetic cardiac stimulation. Note that CNGC are transmembrane proteins that locate to the plasma membrane whereas PKA and EPAC can be targeted to many different intra-cellular locations depending on the identity of the anchor proteins to which they are sequestered. **(B)** β arrestin mediates translocation of PDE4D5 in heart and airway cells following activation of the β2-adrenergic receptor. This action terminates signaling to the cAMP effector proteins, PKA and possibly the EPAC and cyclic nucleotide-gated ion channels. The modular structure of PDE4D5 is depicted demonstrating the N-terminal targeting region, upstream conserved regions that regulate enzyme activity and the catalytic domain.

Key Term

PDEs: Super-family of enzymes that have the unique ability to hydrolyze the cyclic-nucleotides cAMP and cGMP. There are 11 known families (PDE1–11) with PDE4, 7 and 8 being cAMP specific, PDE5, 6 and 9 being cGMP specific and the other five (PDEs 1, 2, 3, 10 and 11) having dual specificity with differing affinities for both types of cyclic nucleotide.

of β-adrenergic stimuli [19]. These gradients are essential for tailoring receptor-specific responses because cAMP effectors such as PKA and EPAC are anchored at specific intracellular sites where they 'read' cAMP concentrations and instigate downstream signaling events. The cAMP gradients themselves could be shaped by discretely positioned PDEs that restrict the free diffusion of the cyclic nucleotide **(Figure 3)** [20] and/or structural impediments and local changes in viscosity [21]. However, it is recognized that it is the equilibrium between cAMP synthesis by AC and the hydrolytic activity of the PDE complement of the cardiac myocyte that controls magnitude and duration of cAMP-dependent signaling processes [22].

In summation, the fidelity of compartmentalized cAMP signals that result in receptor specific physiological changes in cardiac myocytes depend on the position of the enzymes that synthesize the second messenger (AC at the plasma membrane), the localization of the enzymes which hydrolyze cAMP (targeted PDEs) and the anchoring of cAMP-effector proteins (PKA, EPAC) that act to convert 'cAMP currency' into actual cellular responses.

Disregulation of certain signaling responses at these nodes can result in cardiovascular disease and the cAMP signaling system in cardiac cells has long been regarded as an axis that could be targeted as a therapeutic route [23–25]. However, as PKA, EPAC and PDEs are ubiquitous proteins that undertake numerous, unrelated functions in a cell or tissue, active site directed inhibitors, which act nonspecifically to prohibit equally the activity of complete enzyme families, would have an increased chance of inducing serious 'off-target' effects. With this in mind, a more sensible approach would be to target specific protein–protein interactions that underpin the compartmentalization of EPAC, PKA and PDEs. In this way, it should be possible to isolate and displace the activity of a single 'pool' of one of these enzymes so that any treatment would not impinge on the other activities of the enzyme in question. This may combat the action of a hyper- or hypo-activated cAMP-effector enzyme (PKA or EPAC) or compensate for the reduced/increased activity of a mutated PDE or AC. This type of research is in its infancy and the aim of this review is to highlight recent advances and discuss on-going problems in this area.

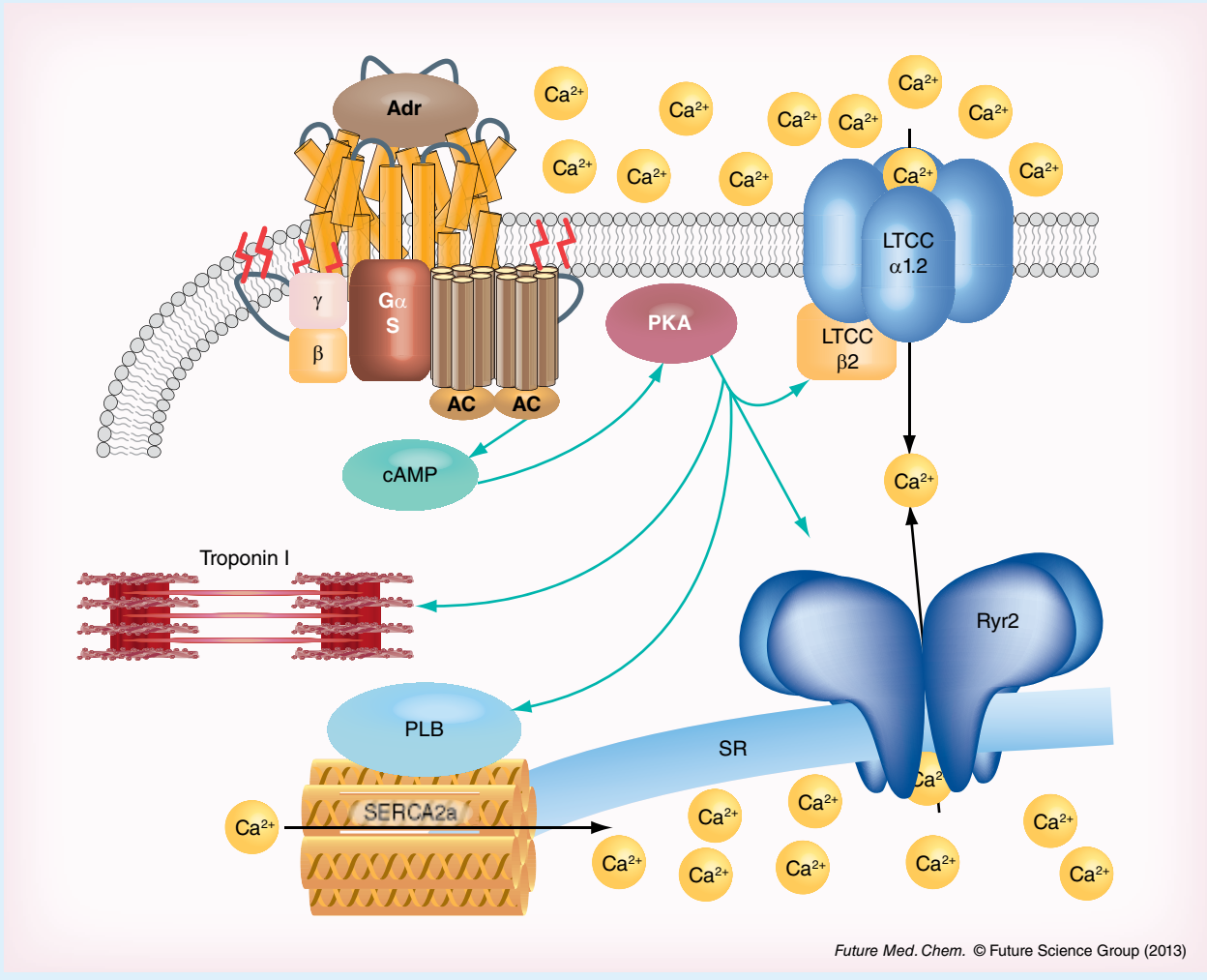

Figure 2. PKA promotes sympathetic stimulation of heart function. β-adrenergic stimulation via adrenaline results in the activation of localized pools of PKA that act to phosphorylate a number of proteins, which orchestrate excitation–contraction coupling. In a coordinated fashion, PKA directly phosphorylates the LTCC, RyR2, PLB and troponin I to promote calcium (Ca^{2+}) influx, calcium release from intracellular sarcoplasmic stores, cardiac muscle contraction and calcium reuptake.
LTCC: L-type calcium channels; SR: sarcoplasmic reticulum.

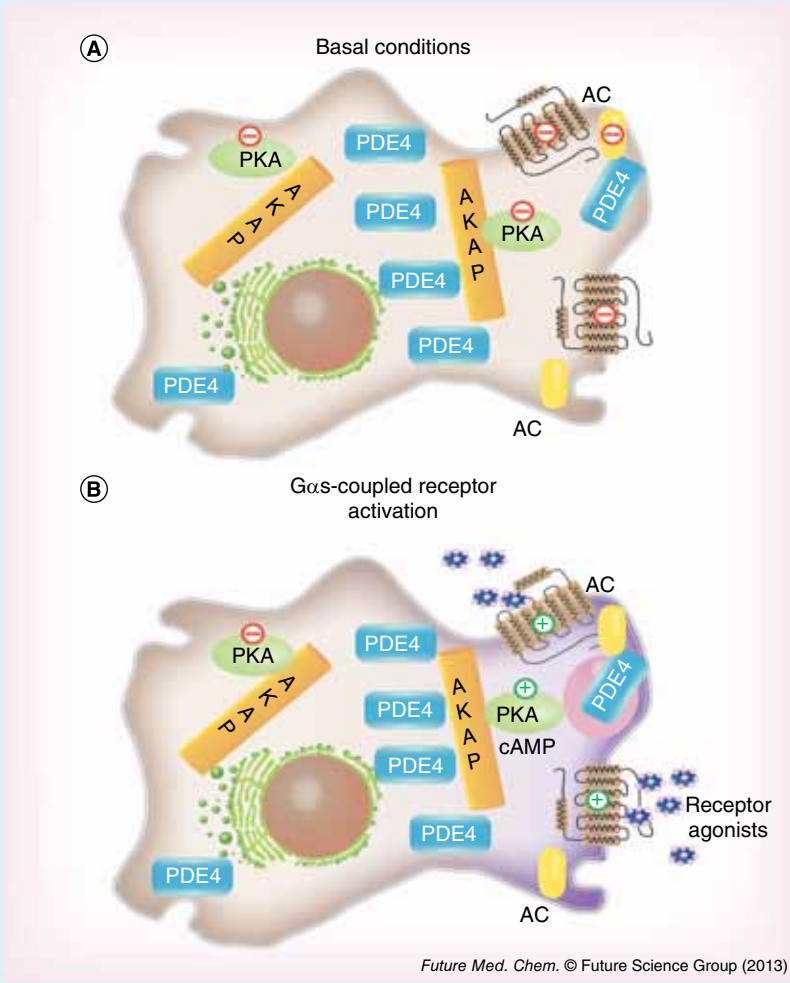

Figure 3. Spatially restricted cAMP gradients are shaped by discretely positioned PDEs. Following addition of β-adrenergic stimuli, receptor specific responses depend on the positioning of localized pools of PDE that shape cAMP gradients (purple) within defined cellular compartments. This action allows fine control of the magnitude and duration of cAMP-dependent signaling processes that depend on the activation of cAMP-effector proteins PKA as demonstrated in this diagram, which 'read' local cAMP concentrations. AKAPs sequester PKA to defined cellular compartments.

Disruption of phosphodiesterase targeting

PDEs have been classed into eleven families (PDE1–11), with PDE5, 6 and 9 being cGMP specific, PDE4, 7 and 8 being cAMP specific and the other five PDEs (PDE1, 2, 3, 10 and 11) having dual specificity with differing affinities for both types of cyclic nucleotide [26]. As the various PDE families are encoded by multiple genes, with alternate splicing sites and alternate promoters, the number of transcripts is large, and this results in the expression of a highly diverse group of enzymes with divergent functions [27].

In this article we focus on the cAMP-specific PDE4 family as it has provided much insight into cAMP signal compartmentalization in cells of the cardiovascular system [22]. PDE4s are encoded by four genes (A, B, C and D) and these give rise to at least 25 different proteins (six PDE4A forms, five PDE4B forms, three PDE4C forms and 11 PDE4D forms) via mRNA splicing and promoter diversity [28]. Indeed, study of this PDE family has provided the paradigm for intracellular targeting of cAMP hydrolysis and this is because all PDE4 isoforms have similar K_{ms} and V_{max} for cAMP hydrolysis, hence their functional role is determined largely by their cellular location, interaction with other signaling proteins and post-translational modification. In keeping with all other PDE families, PDE4s have a complex modular structure, consisting of a conserved catalytic domain, sub-family specific C-terminal domain, dual regulatory domains called upstream conserved region 1 and upstream conserved region 2 together with an isoform specific N-terminal region **(Figure 1)** [28]. Importantly, in the context of this review, the fundamental roles that individual PDE4 isoforms play in tailoring compartmentalized cAMP signals are conferred by the unique localization sequences that are contained within isoform-specific N-terminal regions. This 'post-code' sequence or other localization sequences direct the association of PDE4 family members to a variety of inert scaffolds, lipids or active proteins. These include RACK1 [29], β-arrestin [30], AKAP18 [31], β1-adrenergic receptor [32], immunophillin XAP2 [33], mAKAP [34,35], SRC family tyrosine kinase [36], the dynein complex member Nudel [37], the p75 neurotrophin receptor[38], DISC1 [39], the cardiac IKs potassium channel [40], the PDZ domain containing protein Shank2 [41] and phosphatidic acid [42,43].

It has been known for some time that inhibitors directed against the active site of PDE4 isoforms have great potential for the treatment of a variety of diseases (reviewed in [28,44]). However, in practice, their clinical utility is compromised by side effects that limit maximally tolerated doses [45]. Headache, nausea, emesis and diarrhea are the most commonly reported side effects and these stem from the inhibition of PDE4 isoforms in non-target tissues. Specifically, PDE4D expression is high in an area of the brain known to trigger nausea called the area postrema [45]. Despite the disadvantages of PDE4 inhibitors, one such compound (roflumilast) has recently been approved by the European Commission and US FDA for the

treatment of severe chronic obstructive pulmonary disease [46], however, there is still concern over side effects such as diarrhea, pancreatitis and weight loss associated with taking the drug [47]. One route to aid development of a novel, safer class of PDE4 inhibitor would be to target the protein–protein interactions that underpin the enzyme's localization. The unique, non-redundant roles that PDE4 isoforms play [48] in shaping compartmentalized cAMP signals have only recently been discovered using novel technologies such as siRNA knockdown [49], dominant negative transfection of catalytically dead PDE4 isoforms [50], knockout mice [51] and peptide interference [52]. The first three of these approaches represent powerful techniques for elucidating the functions of anchored PDE4 isoforms, however, they are limited by the fact that they target all subpopulations of the same isoform. This may render investigation of functional outputs problematic for isoforms such as PDE4D5, which have multiple non-redundant functions within the same cell or tissue [20]. The peptide interference approach, in comparison, targets the disruption of PDE4 cellular targeting rather than PDE catalytic activity and, as such, can be used to investigate the function of different 'pools' of the same isoform. The technique relies on accurate peptide mapping of the protein–protein interactions that anchor PDE4 isoforms (or any other protein target) to their scaffolds. Such information has been facilitated by the introduction of peptide array technology, a technique that allows rapid determination of the molecular nature of protein–protein interactions [53]. A library of spotted, immobilized peptides (overlapping peptides up to 25mers, each shifted by five amino acids) of the PDE4 localization sequence can be probed by a purified, recombinant form of the PDE4 scaffolding protein using a simple 'far-western' overlay protocol. Positive spots contain putative binding sequences that can then be used to inform mutagenesis studies on the PDE4 isoform to both verify the sites of interaction and probe for novel PDE4 functions. The identified sequences can also be converted into powerful small peptide agents that have the potential to interfere with protein interactions *in vivo*. This disruption approach has been used extensively in the cAMP signaling field to investigate the purpose of interactions between PDE4 enzymes and the signaling proteins RACK1 [29], β arrestin [29,30] and the cardioprotective chaperone HSP20 [54]. Of these examples, the latter two offer therapeutic potential in the cardiovascular area.

Disruption of the PDE4D5–β arrestin complex

There is now a large body of evidence to suggest that β arrestin, a multifunctional scaffold protein, orchestrates the dynamic redistribution of the PDE4 isoform PDE4D5 in heart and airway cells following activation of the β2-adrenergic receptor [55–60]. Indeed, the involvement of PDE4D5 in the β-adrenergic response constitutes the first example of a biological function ascribed to a single PDE4 isoform and represents the initial realization that although PDE4 isoforms are tethered, that did not mean they were static but instead could be translocated in conjunction with other proteins to sites of high cAMP concentrations within the cell [48]. Thus, the β arrestin–PDE4D5 complex [59] is shuttled to activated β2-adrenergic receptors so that an active pool of PDE4 is recruited to the site of cAMP synthesis to initiate the desensitization process **(FIGURE 1B)** [22]. This action is in synergy with the primary function of β arrestin, which is to sterically hinder signaling between receptors and G_s, initiating a reduction in AC activation and a subsequent decrease in cAMP production [61]. In effect, the β arrestin–PDE4D5 complex serves to orchestrate a dual desensitization process where the 'message' is destroyed at the same time as the receptor's signal to the G-protein is silenced. This, in turn, effectively 'resets' the receptor for another round of agonist challenge. The dynamic movement of PDE4 also downregulates PKA phosphorylation of the β2-adrenergic receptor by the AKAP79–PKA complex [62]. This action promotes switching of β2-adrenergic receptor signaling via AC activation through G_s, to inhibition via G_i and subsequent activation of the ERK–MAP kinase pathway [9].

As eluded to above, a novel approach directed against the targeting of PDE4D5 to specific signalosomes, such as the scaffold protein, β arrestin has been facilitated by the introduction of peptide-array technology. Fine mapping of the β arrestin association site within the unique N-terminal of PDE4D5 has allowed determination of minimal sequences which are essential to promote the interaction between PDE4D5 and β arrestin [29,30] and these have been modeled on the NMR structure of the PDE [52] and verified using yeast-2 hybrid analysis and site-directed mutagenesis [29]. Cell-permeable versions of these sequences have provided 'proof-of-concept'

for this approach as they demonstrated that disturbance of targeted pools of one PDE4 isoform could result in a distinct functional change. In this case, attenuated recruitment of PDE4D5 to the β2-adrenergic receptor leading to a hyperphosphorylation of the receptor after stimulation [52]. Interestingly, disruption of the binding of PDE4D5 to another binding partner, the scaffold RACK1 [63], using a different sequence, led to a very different outcome where PDE4D5 recruitment to the leading edge of cancer cells was attenuated. This action served to inhibit the formation of spreading initiation centers, prevent cell spreading and block cell polarization toward the leading edge of a wound created in a confluent monolayer [64].

Disruption of the PDE4–HSP20 complex

HSPs are a ubiquitous and diverse group of chaperone proteins that are essential for the maintainance of normal cell metabolism. The expression of these proteins is rapidly upregulated during the protective response following periods of cellular stress and this action protects the cell from various kinds of damage [65]. HSPs have been organized into families on account of their molecular weight and function. Small HSPs are the most diverse family and are known for their intrinsic chaperone function as well as the protective effects they confer in neurology and cardiology settings [66]. Recently, one of the ten small HSPs, HSP20 (HSPB6), has been a focus of interest in the field of cardiovascular research. The most widely studied protective capabilities of HSP20 are in the field of cardioprotection, where its actions can be broadly divided into anti-ischemic, anti-apoptotic and antihypertrophic effects (reviewed in [67,68]). Many of the cardioprotective effects underpinned by HSP20 are known to depend on phosphorylation of a PKA site in the N-terminal region of HSP20 (Ser16) [69]. This action is promoted by HSP20s association with AKAP–Lbc [70] and inhibited by its association with PDE4 [71]. Enzymes of the PDE4 family associating with HSP20 act as local cAMP sinks to create areas devoid of the cyclic nucleotide, and thus prevent the phosphorylation of HSP20, unless either the PDE4 is inhibited or the rate of cAMP production rises so as to saturate the pool of PDE4 associated with the chaperone **(Figure 4)** [54]. Thus, chronic β-adrenergic stimulation, with a constant activation of AC and sustained increases in local cAMP levels, saturate the PDE4 pool localized to HSP20, allowing PKA activation and phosphorylation of sequestered HSP20. In times of low basal cAMP, however, the association with PDE4 prevents HSP20 phosphorylation and 'activation' of its cardioprotective abilities. In an attempt to devise an agent that could permanently 'switch on' theses abilities, the site of interaction between HSP20 and PDE4 was mapped using a peptide array. The binding site was found to be within the PDE4 catalytic region and a cell-permeable peptide based on that sequence was shown to disrupt the HSP20–PDE4 complex and induce HSP20 phosphorylation under basal conditions **(Figure 4)** [54]. Pretreatment of cardiac myocytes with the disruptor peptide but not with a scrambled version also afforded protection against the hypertrophic response following chronic β-adrenergic stimulation. Expression levels of the cardiac chaperone, HSP20, have been found to increase in response to hypertrophic stimuli such as aortic constriction and chronic β-adrenergic receptor agonist treatment [72]. Protection against hypertrophy has also been seen in transgenic mice overexpressing HSP20 [73] and this has been linked to a rise in the levels of phospho-HSP20. Moreover, a phospho-mimic mutant of HSP20 (Ser16 to Asp substitution) expressed in isolated adult cardiac myocytes conferred full protection from chronic β-adrenergic stimulation-induced apoptosis, whereas the phospho–null (Ser16 to Ala) did not [74]. It is noteworthy that the same mouse model (overexpressing phospho-null HSP20) was also negative for protection against I/R injury [75] and these results highlight the importance of Ser16 modification in switching HSP20 from a dormant form to the cardioprotective, phosphorylated state. Some experimental success has been achieved with simple cell permeant phospho–peptide analogues encompassing the vital PKA consensus site in the N-terminus. Such peptides may confer protection against a variety of diseases including subarachnoid hemorrhage [76], vasospasm of human umbilical artery [77], keloid scarring [78], airway smooth muscle relaxation [77,79] and platelet aggregation [80]. Clearly, all of these peptide agents have therapeutic potential, with one, namely AZX100 [81] having undergone Phase II clinical trials. However, it is important to point out that the potency of such peptides could be limited by the fact that the sequence from which they are comprised does not contain the α–crystalin domain, a region required for many HSP20 functions. The sequences also

contain an unstable phosphate group, which can be easily targeted by phosphatases and is difficult to mass produce. So, the strategy of inducing endogenously expressed phospho-HSP20 by displacing associated PDE activity, in comparison, should circumvent these problems.

Other medical advantages conferred by HSP20 may be discovered following experimentation into adenoviral gene delivery of HSP20 and viral delivery of shRNA to silence the HSP20 expression. Drug screening of small-molecule libraries could also be a lucrative strategy to harness the therapeutic potential of HSP20. If compounds can be found that mimic the action of peptides discussed above, then these would be easier to convert into useable therapeutics. This approach has been validated by a recent study, which discovered small-molecule modulators of HSP20 signals that caused relaxation of human airway smooth muscle cells and intact tissue *ex vivo* [71].

Disruption of complexes that alter EPAC signaling

Cell-permeable peptides designed to antagonize protein–protein interactions between PDE4 enzymes and EPACs also have recently been demonstrated to afford selective regulation of certain compartmentalized cAMP-mediated events in cells. For instance, PDE4-mediated hydrolysis of a plasma membrane pool of cAMP was recently shown to regulate the ability of EPAC1 to stabilize VECAD–based adherens junctions (AJs) in vascular endothelial cells (VECs) and consequently control paracellular transport of cells or biomacromolecules across the endothelium [5,82,83]. Indeed, inhibition of VEC PDE4 activity increased cAMP, promoted EPAC1-dependent Rap1 activation, strengthened VEC AJs and consequently decreased paracellular VEC transport. Since inhibition of another cAMP-hydrolyzing VEC PDE, namely PDE3, with cilostamide also increased VEC cAMP and activated EPAC1, but did not influence paracellular transport in these cells, the selective action associated with PDE4 inhibition were perhaps best explained through local effects. Consistent with this, VECs AJs were shown to assemble a VECAD-based complex containing β-catenin, p120-catenin, PDE4D, EPAC1 and Rap1. Moreover, treatment of these cells with a stearated EPAC1-based PDE4D displacing peptide promoted Rap1 activation within this complex and increased barrier stability to an extent similar to that afforded by pan-cellular PDE4 inhibition with the PDE4 inhibitor, rolipram **(FIGURE 5)** [84]. When the barrier stabilizing actions of the EPAC1-based PDE4D-displacing peptide were compared with those associated with those resultant from PDE4D knockdown, a critical role for PDE4D in promoting integration of EPAC1 into the VECAD-based complex was observed. Although not yet fully deciphered, clearly this second effect likely involves additional protein–protein interactions between PDE4D and EPAC1, or PDE4D and other members of this signaling complex, which could be targeted.

Notwithstanding the observation that PDE3 inhibitor–induced activation of EPAC1 did not alter VEC permeability, it did impact integrin-based adhesion, migration and tubule formation

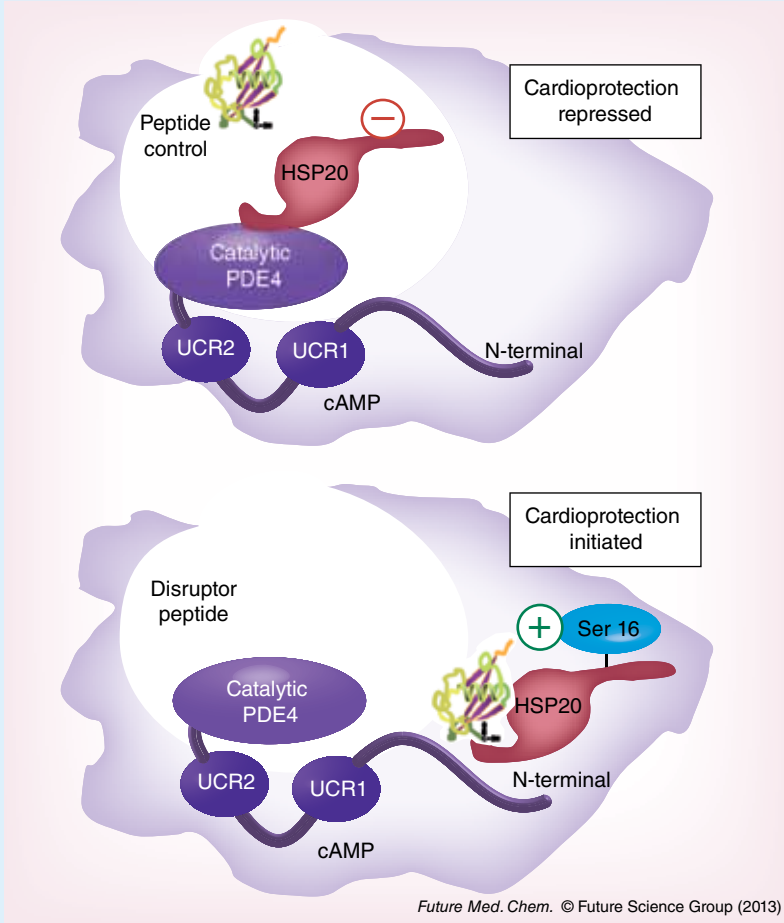

Figure 4. Peptide disruption of the HSP20–PDE4 complex promotes PKA phosphorylation of HSP20 on serine 16 to trigger its cardioprotective action. Peptide array mapping of the HSP20 binding site on PDE4 informed the discovery of a disruptor peptide that could dissociate the complex. This action serves to release the 'brake' on PKA pools that localize with HSP20 (PKA not shown here) and promote the phosphorylation of HSP20 on Ser16. Many of the cardioprotective effects underpinned by HSP20 are known to depend on HSP20 phosphorylation.

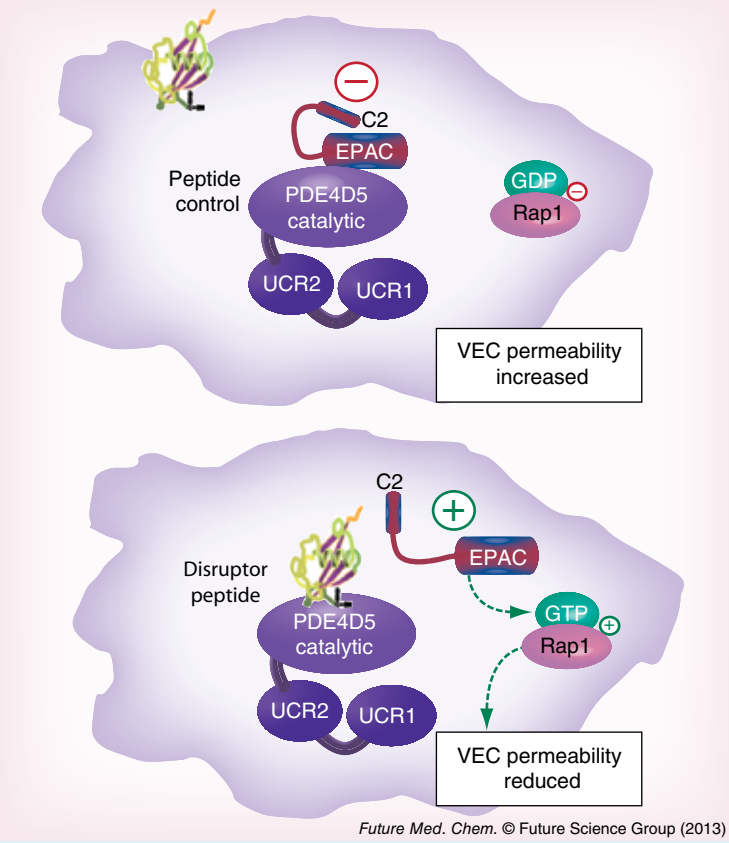

Figure 5. Peptide disruption of the PDE–EPAC complex reduces vascular endothelial cell permeability. A disruptor peptide corresponding to the PDE4 docking site on EPAC disrupted the PDE4–EPAC complex, allowing the activation of EPAC by cAMP. This action promotes EPAC1-dependent Rap1 GTPase activation, strengthens VEC adherens junctions and consequently decreases paracellular VEC transport.
VEC: Vascular endothelial cell.

by VECs [85]. Indeed, these events are coordinated locally in a human VEC complex composed of EPAC1/R-Ras/p84-p110γ and tethered to the plasma membrane by PDE3B [85]. In addition, since PDE3B interacted directly with EPAC1 and with the p84 regulatory domain of PI3Kγ [86,87] via distinct peptide motifs, PDE3B simultaneously tethers each EPAC1 and the p84-regulated form of PI3Kγ to membranes in these cells. Consistent with this, PDE3 inhibition with cilostamide, selective PDE3B knockdown or addition of a PDE3B-based EPAC1-displacing peptide in HAECs each promoted EPAC1-dependent activation of R-Ras, activated the p84-regulated form of PI3Kγ and promoted downstream signaling through both ERK and PKB [85]. These findings, when combined with those obtained using the EPAC1-based PDE4D displacing peptide discussed above, validate the concept that selective regulation of individual cAMP-sensitive cellular events can be achieved with reagents, or drugs, able to selectively disturb the formation of individual cAMP–signalosomes that signal through EPAC1 in cells. This may be important for the development of antihypertophy therapeutics as the EPAC signaling axis has recently been highlighted as a major route for the promotion of the hypertrophic effect of chronic β-adrenergic receptor activation [88,89] via a mechanism involving H-Ras, phospholipase C and HDAC4 translocation [90]. In addition, an EPAC-PDE4D5 signaling axis has recently been recognized as being important in the direction sensing process of migration of cancer cells [91].

Disruption of AKAP complexes

AKAPs are a family of proteins that are instrumental in the maintenance of distinct cAMP signaling compartments in cells, such as those of the heart [92]. They comprise a group of proteins that bind and anchor PKA (a complex consisting of two regulatory subunits [R] that bind to and inhibit two catalytic subunits [C]), thereby acting to sequester and localize the enzyme in the vicinity of its substrates and other signaling proteins that may impinge on PKA function such as phosphatases and other kinases. As such, AKAPs are signaling hubs that organize downstream cardiac signals that are driven by PKA, whilst also serving as crosstalk junctions that allow integration of multiple signaling cascades [93]. AKAPs represent a new and emerging target for drug development for chronic heart failure [23]. A 14–18-amino acid sequence is common to many AKAPs, which allows anchoring to the regulatory subunit dimer of PKA. This series of residues creates an amphipathic α helical structure, which fits into a hydrophobic groove generated by the dimerized regulatory subunits [94]. Over the years, many types of AKAP have been discovered, with the majority being RII-binding AKAPs [95], although some AKAPs with specificity in anchoring of the RI-type subunits have been reported [96]. Additionally, dual specificity AKAPs have been identified, which possess the ability to bind both type I and type II PKAs [97]. As a single AKAP can influence the PKA phosphorylation of many substrates (e.g., AKAP79 directs phosphorylation of ACs 5, 6 and 8 [98,99], the β2-adrenergic receptor [62], β1-adrenergic receptor [100], Kir2.1 potassium channel [101], GluR1 [102], the vallanoid receptor TRPV1 [103] and GABA receptors [104]), disruption of the AKAP–PKA interface has helped in

characterizing a range of functions for specific AKAP types in a variety of cells/tissues. Cell-permeable peptides that displace PKA from AKAPs have helped delineate AKAP function in Tcells [105], pancreatic β cells [106], adrenocortical cells [105], cardiac myocytes [107,108], hippocampal neurons [109] and renal principal cells (reviewed in [110]).

Approximately a third of all known AKAPs are expressed in heart tissue and it is now established that AKAP-scaffolding proteins are vital for the spatial and temporal modulation of cardiac excitation–contraction coupling and the maintenance of healthy cardiac physiology. AKAPs can be linked to Ca^{2+} influx, Ca^{2+} release and re-uptake of Ca^{2+} from sarcoplasmic reticulum calcium stores as well as myocyte repolarization and cardiac remodeling following hypertrophic stimuli (reviewed in [111]). Although our appreciation of AKAP function has dramatically increased over the last decade, little progress has been made at the organism level, as mouse 'knockout' models have failed to shed much light on the functional significance of AKAP-signaling in cardiac disease [111], although some progress has been made with 'knock in' mice in the field of pain management [103]. It has been suggested that this may be due to the fact that genetic silencing of AKAPs may not enable researchers to differentiate between modular and integrative roles that AKAPs take [111] and one way round this problem would be to design peptides or small molecules that selectively target functional domains on AKAPs rather than AKAPs themselves or the AKAP–PKA interface. This method has proved useful in the elucidation of how AKAP15 regulates rapid and efficient PKA phosphorylation of Ca^{2+} channels and increases the L-type calcium current through CaV1,2 channels in isolated cardiac myocytes [112] and skeletal muscle [113]. In these cases, the AKAP associates directly with the C-terminal region of the α subunit of the Ca^{2+} channel via a leucine zipper motif. Peptides that inhibit PKA, displace PKA from AKAP15 or displace AKAP15 from the Ca^{2+} channel, all significantly reduce voltage dependent potentiation of the channel and blocks efficient regulation of L-type Ca^{2+} currents in response to β-adrenergic stimulation. A similar approach was used to highlight the importance of AKAP18δ in calcium reuptake during excitation–contraction coupling [114]. SERCA plays a vital role in Ca^{2+} homeostasis by driving calcium reuptake. SERCA is inhibited constitutively by PLB and this inhibition is released following phosphorylation of PLB by PKA. AKAP18δ coordinates PLB phosphorylation and, hence, orchestrates calcium dynamics in cardiac myocytes. Mapping the AKAP18δ interaction site on PLB allowed invention of a disruptor peptide that could effectively displace the AKAP from PLB and SERCA. This resulted in a significant depression of PLB phosphorylation and slower reuptake of Ca^{2+} into the sarcoplasmic reticulum under basal conditions or following norephinephrine stimulation [114]. Pharmacologic tools that can selectively 'unhook' PKA from a single cardiac substrate could be used to determine the role of signaling scaffolds in the response of failing myocardium to catecholamines and catecholamine antagonists. The AKAP18δ disruptor may also represent a way of manipulating the SERCA–PLB–PKA complex in investigations to combat post-infarction heart failure caused by aberrant PLB function [115,116].

Much effort has been expended in characterizing an AKAP-tethered signaling complex that regulates hypertrophic signaling in cardiac myocytes [117]. mAKAP integrates signals from the cAMP and MAPK cascades by virtue of a unique set of binding partners that include PKA, PDE4D3, the phosphatases, PP2A and calcineurin, EPAC1 and the MAPKs, MEK5 and ERK5 [34], and RSK [118]. Orchestration of signals by mAKAP allows two separate negative feedback loops for cAMP where, in the first instance, PKA phosphorylation of PDE4D3 results in an activation of PDE4 and an increase in affinity of the enzyme for mAKAP. The overall effect is an increase in recruitment of active PDE4 to the mAKAP signalosome, an event that opposes PKA activation. In the second mode, cAMP activates EPAC which then activates Rap1. Rap1 inhibits MEK5 causing a reduction in the inhibitory phosphorylation of PDE4D3 by ERK5 [34]. This increases localized PDE4 activity, again, reducing local cAMP levels and PKA activity. Recently, it has also been demonstrated that the RSK component of the mAKAP hub is known to be important in the maintenance of signaling required for the initiation of hypertrophy in cardiac myocytes [118]. Peptides corresponding to the RSK docking site on mAKAP, inhibited the hypertrophy of cultured myocytes and could represent novel tools that could be developed for the prevention and treatment of heart failure.

Although peptide inhibitors of AKAP function have proved useful in studying the possible roles of these anchor proteins in disease, poor

membrane permeability and lack of oral application severely limit their therapeutic potential. Such problems can be circumvented by the use of small molecules. The only small-molecule inhibitor of AKAP–PKA interactions to be discovered thus far, by high-throughput screening, works allosterically by binding to a region on the regulatory subunits of PKA, inhibiting its association with the AKAP of choice [119]. The compound also activates PKA and can dramatically enhance the phosphorylation of PLB and cardiac troponin-I following β-adrenergic stimulation without affecting the phosphorylation of a variety of other PKA substrates **(Figure 6)**. This dual action serves to increase the contractility of cultured adult rat cardiac myocytes and isolated whole hearts [119]. This new class of molecule can now be tested in animal studies, however, its potential as a compound for the treatment of diseases that are underpinned by aberrant cAMP signaling is unclear.

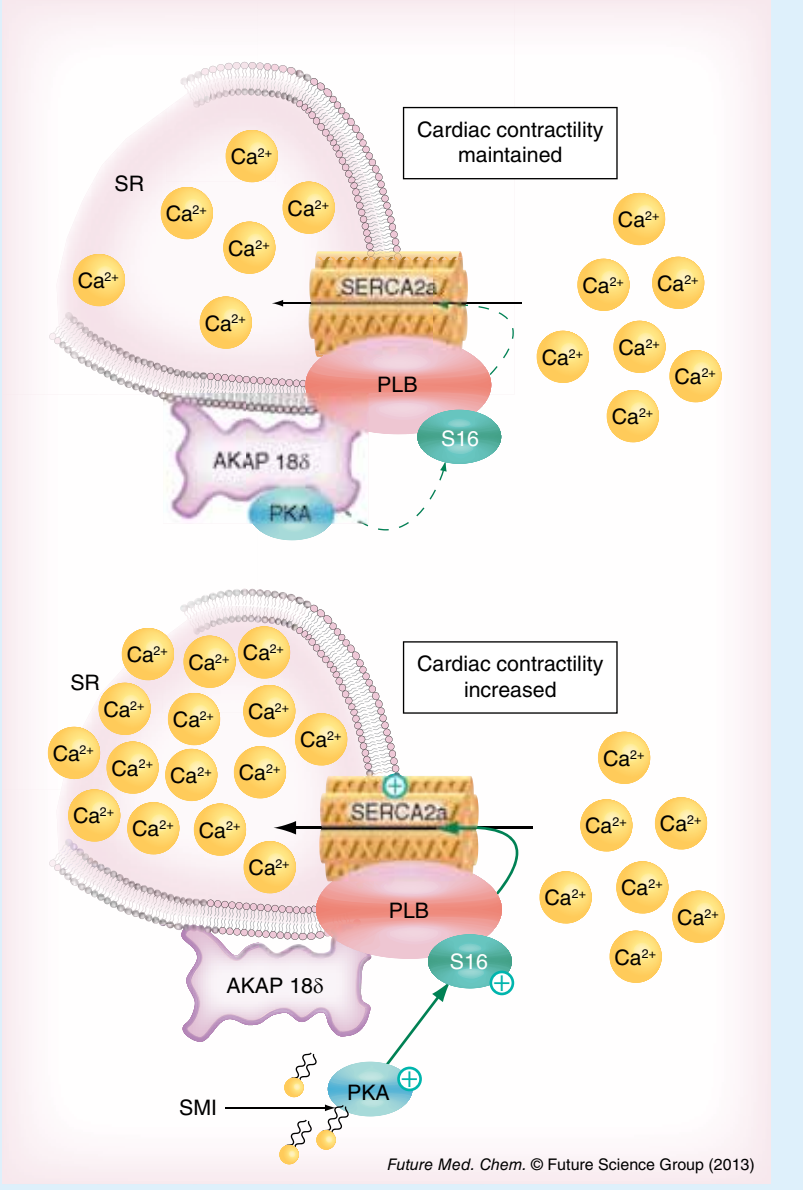

Figure 6. Small-molecule disruptors of the AKAP18δ–PKA interaction promote contractility in cardiac myocytes. Allosteric regulation of PKA regulatory subunits by a small molecule inhibitor releases PKA from AKAP18δ while concomitantly activating the kinase. This action promotes a hyperphosphorylation of PLB diminishing its inhibitory effect on the SERCA. As a result, calcium reuptake into the sarcoplasmic reticulum is enhanced and cardiac contractility is improved.
SM: Small molecule Inhibitor; SR: Sarcoplasmic reticulum.

Future perspective

There is a growing realization that cAMP-signaling in cells occurs in a compartmentalized fashion and that PDEs, cAMP effectors and AKAPs play a central role in coordinating these local effects. Based on this appreciation, a consensus is emerging that selective modulation of the myriad effects coordinated by cAMP in cells will necessitate the use of agents capable of acting within specific compartments. Rising evidence, suggest that disruptor peptides may represent a valid strategy to achieve this goal. Future studies, aimed at determining how individual cAMP-signalosomes are constructed, and in identifying the protein–protein interactions that represent their 'achilles heel' should promote the synthesis and testing of more disruptor peptides and small molecules. These studies will be bolstered by continued biochemical analyses such as those described here and with the implementation of high-resolution structural information in the nature of the protein–protein interactions that underpin their formation. Peptides disrupting defined protein–protein interactions, formulated for direct use, or peptidomimetics based on their structures, are likely to give rise to future biological tools with greater selectivity. This approach may be particularly relevant against discretely targeted PDE isoforms, which modulate the intricate process of excitation–contraction coupling. Recent evidence suggests that spatially confined populations of tethered PDE4s in the heart, generate localized 'sinks' down which cAMP 'disappears' as it is converted into 5′-AMP [20]. So far, the PDE4 compliment associated with the β1-adrenergic receptor [32], β2-adrenergic receptor [59], RyR2 [120], PLB [121] and LTCC [122] have been identified. Development of disruptors against the targeting of these localized PDE4 pools would provide further insight into the non-redundant functions of

these enzymes and provide novel routes of development for therapeutics that improve cardiac function.

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Executive summary

- cAMP signaling in cells occurs in a compartment-specific manner.
- PDEs play a critical role in establishing and maintaining the signaling complexes which coordinate this compartmentalization.
- While a number of PDE inhibitors have found utility in the clinic, since these agents act by competing cAMP binding at the active site, they represent pan-compartment inhibitors and do not allow compartment-selective actions.
- Disruptor peptides (DPs) designed to compete the protein–protein interactions which bring together the individual components of the signaling complexes involved in the propagation of cAMP signals, represent a modality through which compartment-selective actions can be achieved.
- Several DPs that markedly and specifically impact cellular functions by inhibiting the formation of intracellular signaling complexes have been developed.
- Since DPs specifically inhibit binding between proteins in specific cAMP-signalosomes, these agents will allow regulation of cAMP-mediated events selectively within individual cAMP signalosome-based compartments and, thus, will provide increased selectivity over that offered by agents that do not discriminate between these compartments but rather affect global cAMP signaling.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Blumenthal SA. Earl Sutherland (1915–1975) and the discovery of cyclic AMP. *Perspect Biol. Med*. 55(2), 236–249 (2012).

2 Beavo JA, Brunton LL. Cyclic nucleotide research – still expanding after half a century. *Nat. Rev. Mol. Cell Biol.* 3(9), 710–718 (2002).

▪▪ **Comprehensive and informative record of the major breakthroughs in cyclic nucleotide research throughout the last 50 years.**

3 Serezani CH, Ballinger MN, Aronoff DM, Peters-Golden M. Cyclic AMP: master regulator of innate immune cell function. *Am. J. Respir. Cell Mol. Biol.* 39(2), 127–132 (2008).

4 Stork PJ, Schmitt JM. Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. *Trends Cell Biol*. 12(6), 258–266 (2002).

5 Yamamizu K, Yamashita JK. Roles of cyclic adenosine monophosphate signaling in endothelial cell differentiation and arterial-venous specification during vascular development. *Circ. J*. 75(2), 253–260 (2011).

6 Alberini CM, Ghirardi M, Huang YY, Nguyen PV, Kandel ER. A molecular switch for the consolidation of long-term memory: cAMP-inducible gene expression. *Ann. NY Acad. Sci*. 758, 261–286 (1995).

7 Holz GG, Heart E, Leech CA. Synchronizing Ca^{2+} and cAMP oscillations in pancreatic beta-cells: a role for glucose metabolism and GLP-1 receptors? Focus on 'regulation of cAMP dynamics by Ca^{2+} and G protein-coupled receptors in the pancreatic beta-cell: a computational approach'. *Am. J. Physiol. Cell. Physiol.* 294(1), C4–C6 (2008).

8 Lissandron V, Zaccolo M. Compartmentalized cAMP/PKA signalling regulates cardiac excitation-contraction coupling. *J. Muscle Res. Cell Motil.* 27(5–7), 399–403 (2006).

9 Baillie GS, Sood A, McPhee I *et al.* Beta-arrestin–mediated PDE4 cAMP phosphodiesterase recruitment regulates beta-adrenoceptor switching from Gs to Gi. *Proc. Natl Acad. Sci. USA* 100(3), 940–945 (2003).

10 Bers DM. Cardiac excitation-contraction coupling. *Nature*, 415(6868), 198–205 (2002).

11 Li L, Desantiago J, Chu G, Kranias EG, Bers DM. Phosphorylation of phospholamban and troponin I in beta-adrenergic–induced acceleration of cardiac relaxation. *Am. J. Physiol. Heart Circ. Physiol*. 278(3), H769–H779 (2000).

12 Pereira L, Ruiz-Hurtado G, Morel E *et al.* Epac enhances excitation-transcription coupling in cardiac myocytes. *J. Mol. Cell. Cardiol*. 52(1), 283–291(2012).

13 Oestreich EA, Wang H, Malik S *et al.* Epac-mediated activation of phospholipase C(epsilon) plays a critical role in beta-adrenergic receptor-dependent enhancement of Ca^{2+} mobilization in cardiac myocytes. *J. Biol. Chem*. 282(8), 5488–5495 (2007).

14 Kraus-Friedmann N. Cyclic nucleotide–gated channels in non-sensory organs. *Cell Calcium* 27(3), 127–138 (2000).

15 Hayes JS, Brunton LL, Mayer SE. Selective activation of particulate cAMP–dependent protein kinase by isoproterenol and prostaglandin E1. *J. Biol. Chem*. 255(11), 5113–5119 (1980).

16 Brunton LL, Hayes JS, Mayer SE. Functional compartmentation of cyclic AMP and protein kinase in heart. *Adv. Cyclic Nucleotide Res*. 14, 391–397 (1981).

▪▪ **First indication that compartmentalisation of the cAMP signalling system underpinned specificity of cellular response to different receptor agonists.**

17 Welch EJ, Jones BW, Scott JD. Networking with AKAPs: context–dependent regulation of anchored enzymes. *Mol. Interv*. 10(2), 86–97 (2010).

18 Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu. Rev. Biochem*. 76, 481–511 (2007).

19 Zaccolo M, Pozzan T. Discrete microdomains with high concentration of cAMP in stimulated rat neonatal cardiac myocytes. *Science* 295(5560), 1711–1715 (2002).

20 Baillie GS. Compartmentalized signalling: spatial regulation of cAMP by the action of

compartmentalized phosphodiesterases. *FEBS J.* 276(7), 1790–1799 (2009).

21 Feinstein WP, Zhu B, Leavesley SJ, Sayner SL, Rich TC. Assessment of cellular mechanisms contributing to cAMP compartmentalization in pulmonary microvascular endothelial cells. *Am. J. Physiol. Cell. Physiol.* 302(6), C839–C852 (2012).

22 Houslay MD, Baillie GS, Maurice DH. cAMP-Specific phosphodiesterase-4 enzymes in the cardiovascular system: a molecular toolbox for generating compartmentalized cAMP signaling. *Circ. Res.* 100(7), 950–966 (2007).

23 Troger J, Moutty MC, Skroblin P, Klussmann E. A-kinase anchoring proteins as potential drug targets. *Br. J. Pharmacol.* 166(2), 420–433 (2012).

24 Lugnier C. Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. *Pharmacol. Ther.* 109(3), 366–398 (2006).

25 Bos JL. Epac proteins: multi-purpose cAMP targets. *Trends Biochem. Sci.* 31(12), 680–686 (2006).

▪ **Compelling overview of the discovery and function of EPAC proteins.**

26 Houslay MD, Milligan G. Tailoring cAMP-signalling responses through isoform multiplicity. *Trends Biochem. Sci.* 22(6), 217–224 (1997).

27 Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol. Rev.* 58(3), 488–520 (2006).

28 Houslay MD, Schafer P, Zhang KY. Keynote review: phosphodiesterase-4 as a therapeutic target. *Drug Discov. Today.* 10(22), 1503–1519 (2005).

29 Bolger GB, Baillie GS, Li X *et al.* Scanning peptide array analyses identify overlapping binding sites for the signalling scaffold proteins, beta-arrestin and RACK1, in cAMP-specific phosphodiesterase PDE4D5. *Biochem. J.* 398(1), 23–36 (2006).

30 Bolger GB, McCahill A, Huston E *et al.* The unique amino-terminal region of the PDE4D5 cAMP phosphodiesterase isoform confers preferential interaction with beta-arrestins. *J. Biol. Chem.* 278(49), 49230–49238 (2003).

31 Stefan E, Wiesner B, Baillie GS *et al.* Compartmentalization of cAMP-dependent signaling by phosphodiesterase-4D is involved in the regulation of vasopressin-mediated water reabsorption in renal principal cells. *J. Am. Soc. Nephrol.* 18(1), 199–212 (2007).

32 Richter W, Day P, Agrawal R *et al.* Signaling from beta1- and beta2-adrenergic receptors is defined by differential interactions with PDE4. *EMBO J.* 27(2), 384–393 (2008).

33 Bolger GB, Peden AH, Steele MR *et al.* Attenuation of the activity of the cAMP-specific phosphodiesterase PDE4A5 by interaction with the immunophilin XAP2. *J. Biol. Chem.* 278(35), 33351–33363 (2003).

34 Dodge-Kafka KL, Soughayer J, Pare GC *et al.* The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways. *Nature* 437(7058), 574–578 (2005).

35 Carlisle Michel JJ, Dodge KL, Wong W, Mayer NC, Langeberg LK, Scott JD. PKA-phosphorylation of PDE4D3 facilitates recruitment of the mAKAP signalling complex. *Biochem. J.* 381(Pt 3), 587–592 (2004).

36 Beard MB, O'Connell JC, Bolger GB, Houslay MD. The unique N-terminal domain of the cAMP phosphodiesterase PDE4D4 allows for interaction with specific SH3 domains. *FEBS Lett.* 460(1), 173–177 (1999).

37 Collins DM, Murdoch H, Dunlop AJ *et al.* Ndel1 alters its conformation by sequestering cAMP-specific phosphodiesterase-4D3 (PDE4D3) in a manner that is dynamically regulated through Protein Kinase A (PKA). *Cell. Signal.* 20(12), 2356–2369 (2008).

38 Sachs BD, Baillie GS, McCall JR *et al.* p75 neurotrophin receptor regulates tissue fibrosis through inhibition of plasminogen activation via a PDE4/cAMP/PKA pathway. *J. Cell Biol.* 177(6), 1119–1132 (2007).

39 Millar JK, Pickard BS, Mackie S *et al.* DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science* 310(5751), 1187–1191 (2005).

40 Terrenoire C, Houslay MD, Baillie GS, Kass RS. The cardiac IKs potassium channel macromolecular complex includes the phosphodiesterase PDE4D3. *J. Biol. Chem.* 284(14), 9140–9146 (2009).

41 Lee JH, Richter W, Namkung W *et al.* Dynamic regulation of cystic fibrosis transmembrane conductance regulator by competitive interactions of molecular adaptors. *J. Biol. Chem.* 282(14), 10414–10422 (2007).

42 Baillie GS, Huston E, Scotland G *et al.* TAPAS-1, a novel microdomain within the unique N-terminal region of the PDE4A1 cAMP-specific phosphodiesterase that allows rapid, Ca^{2+}-triggered membrane association with selectivity for interaction with phosphatidic acid. *J. Biol. Chem.* 277(31), 28298–28309 (2002).

43 Grange M, Sette C, Cuomo M *et al.* The cAMP-specific phosphodiesterase PDE4D3 is regulated by phosphatidic acid binding. Consequences for cAMP signaling pathway and characterization of a phosphatidic acid binding site. *J. Biol. Chem.* 275(43), 33379–33387 (2000).

44 Francis SH, Houslay MD, Conti M. Phosphodiesterase inhibitors: factors that influence potency, selectivity, and action. *Handb. Exp. Pharmacol.* 204, 47–84 (2011).

45 Zhang KY, Ibrahim PN, Gillette S, Bollag G. Phosphodiesterase-4 as a potential drug target. *Expert Opin. Ther. Targets.* 9(6), 1283–1305 (2005).

46 Fabbri LM, Beghe B, Yasothan U, Kirkpatrick P. Roflumilast. *Nat. Rev. Drug Discov.* 9(10), 761–762 (2010).

47 Gupta S. Side-effects of roflumilast. *Lancet* 379(9817), 710–711; Author reply 711–712 (2012).

48 Lynch MJ, Baillie GS, Houslay MD. cAMP-specific phosphodiesterase-4D5 (PDE4D5) provides a paradigm for understanding the unique non-redundant roles that PDE4 isoforms play in shaping compartmentalized cAMP cell signalling. *Biochem. Soc. Trans.* 35(Pt 5), 938–941 (2007).

49 Lynch MJ, Baillie GS, Mohamed A *et al.* RNA silencing identifies PDE4D5 as the functionally relevant cAMP phosphodiesterase interacting with beta-arrestin to control the protein kinase A/AKAP79-mediated switching of the beta2-adrenergic receptor to activation of ERK in HEK293B2 cells. *J. Biol. Chem.* 280(39), 33178–33189 (2005).

50 McCahill A, McSorley T, Huston E *et al.* In resting COS1 cells a dominant negative approach shows that specific, anchored PDE4 cAMP phosphodiesterase isoforms gate the activation, by basal cyclic AMP production, of AKAP-tethered protein kinase A type II located in the centrosomal region. *Cell. Signal.* 17(9), 1158–1173 (2005).

51 Bruss MD, Richter W, Horner K, Jin SL, Conti M. Critical role of PDE4D in beta2-adrenoceptor-dependent cAMP signaling in mouse embryonic fibroblasts. *J. Biol. Chem.* 283(33), 22430–22442 (2008).

52 Smith KJ, Baillie GS, Hyde EI *et al.* 1H NMR structural and functional characterisation of a cAMP-specific phosphodiesterase-4D5 (PDE4D5) N-terminal region peptide that disrupts PDE4D5 interaction with the signalling scaffold proteins, beta-arrestin and RACK1. *Cell. Signal.* 19(12), 2612–2624 (2007).

53 Frank R. High-density synthetic peptide microarrays: emerging tools for functional

genomics and proteomics. *Comb. Chem. High Throughput Screen.* 5(6), 429–440 (2002).

54 Sin YY, Edwards HV, Li X *et al.* Disruption of the cyclic AMP phosphodiesterase-4 (PDE4)-HSP20 complex attenuates the beta-agonist induced hypertrophic response in cardiac myocytes. *J. Mol. Cell. Cardiol.* 50(5), 872–883 (2011).

55 Le Jeune IR, Shepherd M, Van Heeke G, Houslay MD, Hall IP. Cyclic AMP-dependent transcriptional up-regulation of phosphodiesterase 4D5 in human airway smooth muscle cells. Identification and characterization of a novel PDE4D5 promoter. *J. Biol. Chem.* 277(39), 35980–35989 (2002).

56 Mongillo M, McSorley T, Evellin S *et al.* Fluorescence resonance energy transfer-based analysis of cAMP dynamics in live neonatal rat cardiac myocytes reveals distinct functions of compartmentalized phosphodiesterases. *Circ. Res.* 95(1), 67–75 (2004).

57 Willoughby D, Baillie GS, Lynch MJ, Ciruela A, Houslay MD, Cooper DM. Dynamic regulation, desensitization, and cross-talk in discrete subcellular microdomains during beta2-adrenoceptor and prostanoid receptor cAMP signaling. *J. Biol. Chem.* 282(47), 34235–34249 (2007).

58 Trian T, Burgess JK, Niimi K *et al.* Beta2-agonist induced cAMP is decreased in asthmatic airway smooth muscle due to increased PDE4D. *PLoS ONE* 6(5), e20000 (2011).

59 Perry SJ, Baillie GS, Kohout TA *et al.* Targeting of cyclic AMP degradation to beta 2-adrenergic receptors by beta-arrestins. *Science* 298(5594), 834–836 (2002).

▪ **First indication that PDE4 activity could translocate with β arrestin proteins to aid the desensitization of β 2-adrenergic receptors.**

60 Billington CK, Le Jeune IR, Young KW, Hall IP. A major functional role for phosphodiesterase 4D5 in human airway smooth muscle cells. *Am. J. Respir. Cell. Mol. Biol.* 38, 1–7 (2008).

61 Shenoy SK, Lefkowitz RJ. beta-arrestin-mediated receptor trafficking and signal transduction. *Trends Pharmacol. Sci.* 32(9), 521–533 (2011).

62 Fraser ID, Cong M, Kim J *et al.* Assembly of an A kinase-anchoring protein-beta(2)-adrenergic receptor complex facilitates receptor phosphorylation and signaling. *Curr. Biol.* 10(7), 409–412 (2000).

63 Yarwood SJ, Steele MR, Scotland G, Houslay MD, Bolger GB. The RACK1 signaling scaffold protein selectively interacts with the cAMP-specific phosphodiesterase PDE4D5 isoform. *J. Biol. Chem.* 274(21), 14909–14917 (1999).

64 Serrels B, Sandilands E, Serrels A *et al.* A complex between FAK, RACK1, and PDE4D5 controls spreading initiation and cancer cell polarity. *Curr.Biol.* 20(12), 1086–1092 (2010).

65 Aufricht C. HSP: helper, suppressor, protector. *Kidney Int.* 65(2), 739–740 (2004).

66 Mymrikov EV, Seit-Nebi AS, Gusev NB. Large potentials of small heat shock proteins. *Physiol. Rev.* 91(4), 1123–1159 (2011).

67 Fan GC, Kranias EG. Small heat shock protein 20 (HspB6) in cardiac hypertrophy and failure. *J. Mol. Cell. Cardiol.* 51(4), 574–577 (2010).

68 Edwards HV, Cameron RT, Baillie GS. The emerging role of HSP20 as a multifunctional protective agent. *Cell. Signal.* 23(9), 1447–1454 (2011).

69 Edwards HV, Scott JD, Baillie GS. PKA phosphorylation of the small heat-shock protein Hsp20 enhances its cardioprotective effects. *Biochem. Soc. Trans.* 40(1), 210–214 (2012).

70 Edwards HV, Scott JD, Baillie GS. The A-kinase-anchoring protein AKAP-Lbc facilitates cardioprotective PKA phosphorylation of Hsp20 on Ser16. *Biochem J.* 446(3), 437–443 (2012).

71 An SS, Askovich PS, Zarembinski TI *et al.* A novel small molecule target in human airway smooth muscle for potential treatment of obstructive lung diseases: a staged high-throughput biophysical screening. *Respir. Res.* 12(1), 8 (2011).

72 Willis MS, Patterson C. Hold me tight: role of the heat shock protein family of chaperones in cardiac disease. *Circulation* 122(17), 1740–1751 (2010).

73 Fan GC, Yuan Q, Song G *et al.* Small heat-shock protein Hsp20 attenuates beta-agonist-mediated cardiac remodeling through apoptosis signal-regulating kinase 1. *Circ. Res.* 99(11), 1233–1242 (2006).

74 Fan GC, Chu G, Mitton B, Song Q, Yuan Q, Kranias EG. Small heat-shock protein Hsp20 phosphorylation inhibits beta-agonist-induced cardiac apoptosis. *Circ. Res.* 94(11), 1474–1482 (2004).

75 Fan GC, Ren X, Qian J *et al.* Novel cardioprotective role of a small heat-shock protein, Hsp20, against ischemia/reperfusion injury. *Circulation.* 111(14), 1792–1799 (2005).

76 Dreiza CM, Brophy CM, Komalavilas P *et al.* Transducible heat shock protein 20 (HSP20) phosphopeptide alters cytoskeletal dynamics. *FASEB J.* 19(2), 261–263 (2005).

77 Flynn CR, Brophy CM, Furnish EJ *et al.* Transduction of phosphorylated heat shock-related protein 20, HSP20, prevents vasospasm of human umbilical artery smooth muscle. *J. Appl. Physiol.* 98(5), 1836–1845 (2005).

78 Dreiza CM, Komalavilas P, Furnish EJ *et al.* The small heat shock protein, HSPB6, in muscle function and disease. *Cell Stress Chaperones*, 15(1), 1–11 (2009).

79 Komalavilas P, Penn RB, Flynn CR *et al.* The small heat shock-related protein, HSP20, is a cAMP-dependent protein kinase substrate that is involved in airway smooth muscle relaxation. *Am. J. Physiol. Lung. Cell Mol. Physiol.* 294(1), L69–L78 (2008).

80 Kanno Y, Matsuno H. The possibility of novel antiplatelet peptides: the physiological effects of low molecular weight HSPs on platelets. *Curr. Pharm. Des.* 12(7), 887–892 (2006).

81 Lopes LB, Furnish EJ, Komalavilas P *et al.* Cell permeant peptide analogues of the small heat shock protein, HSP20, reduce TGF-beta1-induced CTGF expression in keloid fibroblasts. *J. Invest. Dermatol.* 129(3), 590–598 (2009).

82 Fantidis P. The role of intracellular 3′5′-cyclic adenosine monophosphate (cAMP) in atherosclerosis. *Curr. Vasc. Pharmacol.* 8(4), 464–472 (2009).

83 Sayner SL. Emerging themes of cAMP regulation of the pulmonary endothelial barrier. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 300(5), L667–L678 (2011).

84 Rampersad SN, Ovens JD, Huston E *et al.* Cyclic AMP phosphodiesterase 4D (PDE4D) Tethers EPAC1 in a vascular endothelial cadherin (VE-Cad)-based signaling complex and controls cAMP-mediated vascular permeability. *J. Biol. Chem.* 285(44), 33614–33622 (2010).

85 Wilson LS, Baillie GS, Pritchard LM *et al.* A phosphodiesterase 3B-based signaling complex integrates exchange protein activated by cAMP 1 and phosphatidylinositol 3-kinase signals in human arterial endothelial cells. *J. Biol. Chem.* 286(18), 16285–16296 (2011).

86 Patrucco E, Notte A, Barberis L *et al.* PI3Kgamma modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. *Cell* 118(3), 375–387 (2004).

87 Voigt P, Dorner MB, Schaefer M. Characterization of p87PIKAP, a novel regulatory subunit of phosphoinositide 3-kinase gamma that is highly expressed in heart and interacts with PDE3B. *J. Biol. Chem.* 281(15), 9977–9986 (2006).

88 Metrich M, Lucas A, Gastineau M *et al.* Epac mediates beta-adrenergic receptor-induced

cardiomyocyte hypertrophy. *Circ. Res.* 102(8), 959–965 (2008).

89 Metrich M, Morel E, Berthouze M *et al.* Functional characterization of the cAMP-binding proteins Epac in cardiac myocytes. *Pharmacol. Rep.* 61(1), 146–153 (2009).

90 Metrich M, Laurent AC, Breckler M *et al.* Epac activation induces histone deacetylase nuclear export via a Ras-dependent signalling pathway. *Cell. Signal.* 22(10), 1459–1468 (2010).

91 Serrels B, Sandilands E, Frame MC. Signaling of the direction-sensing FAK/RACK1/PDE4D5 complex to the small GTPase Rap1. *Small GTPases* 2(1), 54–61 (2011).

92 McConnachie G, Langeberg LK, Scott JD. AKAP signaling complexes: getting to the heart of the matter. *Trends Mol. Med.* 12(7), 317–323 (2006).

93 Dodge-Kafka KL, Langeberg L, Scott JD. Compartmentation of cyclic nucleotide signaling in the heart: the role of A-kinase anchoring proteins. *Circ. Res.* 98(8), 993–1001 (2006).

94 Banky P, Newlon MG, Roy M, Garrod S, Taylor SS, Jennings PA. Isoform-specific differences between the type Ialpha and IIalpha cyclic AMP-dependent protein kinase anchoring domains revealed by solution NMR. *J. Biol. Chem.* 275(45), 35146–35152 (2000).

95 Stokka AJ, Gesellchen F, Carlson CR, Scott JD, Herberg FW, Tasken K. Characterization of A-kinase-anchoring disruptors using a solution-based assay. *Biochem J.* 400(3), 493–499 (2006).

96 Angelo R, Rubin CS. Molecular characterization of an anchor protein (AKAPCE) that binds the RI subunit (RCE) of type I protein kinase A from *Caenorhabditis elegans*. *J. Biol. Chem.* 273(23), 14633–14643 (1998).

97 Huang LJ, Durick K, Weiner JA, Chun J, Taylor SS. Identification of a novel protein kinase A anchoring protein that binds both type I and type II regulatory subunits. *J. Biol. Chem.* 272(12), 8057–8064 (1997).

98 Bauman AL, Soughayer J, Nguyen BT *et al.* Dynamic regulation of cAMP synthesis through anchored PKA-adenylyl cyclase V/VI complexes. *Mol. Cell* 23(6), 925–931 (2006).

99 Willoughby D, Masada N, Wachten S *et al.* AKAP79/150 interacts with AC8 and regulates Ca^{2+}-dependent cAMP synthesis in pancreatic and neuronal systems. *J. Biol. Chem.* 285(26), 20328–20342 (2010).

100 Gardner LA, Tavalin SJ, Goehring AS, Scott JD, Bahouth SW. AKAP79-mediated targeting of the cyclic AMP-dependent protein kinase to the beta1-adrenergic receptor promotes recycling and functional resensitization of the receptor. *J. Biol. Chem.* 281(44), 33537–33553 (2006).

101 Dart C, Leyland ML. Targeting of an A kinase-anchoring protein, AKAP79, to an inwardly rectifying potassium channel, Kir2.1. *J. Biol. Chem.* 276(23), 20499–20505 (2001).

102 Tavalin SJ, Colledge M, Hell JW, Langeberg LK, Huganir RL, Scott JD. Regulation of GluR1 by the A-kinase anchoring protein 79 (AKAP79) signaling complex shares properties with long-term depression. *J. Neurosci.* 22(8), 3044–3051 (2002).

103 Schnizler K, Shutov LP, Van Kanegan MJ *et al.* Protein kinase A anchoring via AKAP150 is essential for TRPV1 modulation by forskolin and prostaglandin E2 in mouse sensory neurons. *J. Neurosci.* 28(19), 4904–4917 (2008).

104 Brandon NJ, Jovanovic JN, Colledge M *et al.* A-kinase anchoring protein 79/150 facilitates the phosphorylation of GABA(A) receptors by cAMP-dependent protein kinase via selective interaction with receptor beta subunits. *Mol. Cell. Neurosci.* 22(1), 87–97 (2003).

105 Carlson CR, Lygren B, Berge T *et al.* Delineation of type I protein kinase A-selective signaling events using an RI anchoring disruptor. *J. Biol. Chem.* 281(30), 21535–21545 (2006).

106 Faruque OM, Le-Nguyen D, Lajoix AD *et al.* Cell-permeable peptide-based disruption of endogenous PKA-AKAP complexes: a tool for studying the molecular roles of AKAP-mediated PKA subcellular anchoring. *Am. J. Physiol. Cell Physiol.* 296(2), C306–C316 (2009).

107 McConnell BK, Popovic Z, Mal N *et al.* Disruption of protein kinase A interaction with A-kinase-anchoring proteins in the heart *in vivo*: effects on cardiac contractility, protein kinase A phosphorylation, and troponin I proteolysis. *J. Biol. Chem.* 284(3), 1583–1592 (2009).

108 Patel HH, Hamuro LL, Chun BJ *et al.* Disruption of protein kinase A localization using a trans-activator of transcription (TAT)-conjugated A-kinase-anchoring peptide reduces cardiac function. *J. Biol. Chem.* 285(36), 27632–27640 (2010).

109 Gold MG, Lygren B, Dokurno P *et al.* Molecular basis of AKAP specificity for PKA regulatory subunits. *Mol. Cell* 24(3), 383–395 (2006).

110 Hundsrucker C, Klussmann E. Direct AKAP-mediated protein–protein interactions as potential drug targets. *Handb. Exp. Pharmacol.* 186, 483–503 (2008).

111 Mauban JR, O'Donnell M, Warrier S, Manni S, Bond M. AKAP-scaffolding proteins and regulation of cardiac physiology. *Physiology* 24, 78–87 (2009).

112 Hulme JT, Lin TW, Westenbroek RE, Scheuer T, Catterall WA. Beta-adrenergic regulation requires direct anchoring of PKA to cardiac CaV1.2 channels via a leucine zipper interaction with A kinase-anchoring protein 15. *Proc. Natl Acad. Sci. USA* 100(22), 13093–13098 (2003).

113 Hulme JT, Ahn M, Hauschka SD, Scheuer T, Catterall WA. A novel leucine zipper targets AKAP15 and cyclic AMP-dependent protein kinase to the C terminus of the skeletal muscle Ca^{2+} channel and modulates its function. *J. Biol. Chem.* 277(6), 4079–4087 (2002).

114 Lygren B, Carlson CR, Santamaria K *et al.* AKAP complex regulates Ca^{2+} re-uptake into heart sarcoplasmic reticulum. *EMBO Rep.* 8(11), 1061–1067 (2007).

115 Schmitt JP, Kamisago M, Asahi M *et al.* Dilated cardiomyopathy and heart failure caused by a mutation in phospholamban. *Science* 299(5611), 1410–1413 (2003).

116 Haghighi K, Schmidt AG, Hoit BD *et al.* Superinhibition of sarcoplasmic reticulum function by phospholamban induces cardiac contractile failure. *J. Biol. Chem.* 276(26), 24145–24152 (2001).

117 Bauman AL, Michel JJ, Henson E, Dodge-Kafka KL, Kapiloff MS. The mAKAP signalosome and cardiac myocyte hypertrophy. *IUBMB Life* 59(3), 163–169 (2007).

118 Li J, Kritzer MD, Carlisle Michel JJ *et al.* Anchored p90 ribosomal S6 kinase 3 is required for cardiac myocyte hypertrophy. *Circ. Res.* 112(1), 128–139 (2012).

119 Christian F, Szaszak M, Friedl S *et al.* Small molecule AKAP-protein kinase A (PKA) interaction disruptors that activate PKA interfere with compartmentalized cAMP signaling in cardiac myocytes. *J. Biol. Chem.* 286(11), 9079–9096 (2011).

120 Lehnart SE, Wehrens XH, Reiken S *et al.* Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. *Cell* 123(1), 25–35 (2005).

121 Beca S, Helli PB, Simpson JA *et al.* Phosphodiesterase 4D regulates baseline sarcoplasmic reticulum Ca^{2+} release and cardiac contractility, independently of L-type Ca^{2+} current. *Circ. Res.* 109(9), 1024–1030 (2011).

122 Leroy J, Richter W, Mika D *et al.* Phosphodiesterase 4B in the cardiac L-type Ca(2)(+) channel complex regulates Ca(2)(+) current and protects against ventricular arrhythmias in mice. *J. Clin. Invest.* 121(7), 2651–2661 (2011).